Assimilation and depuration of dietary methylmercury in adult zebrafish (*Danio rerio*) and the influence of dietary selenium

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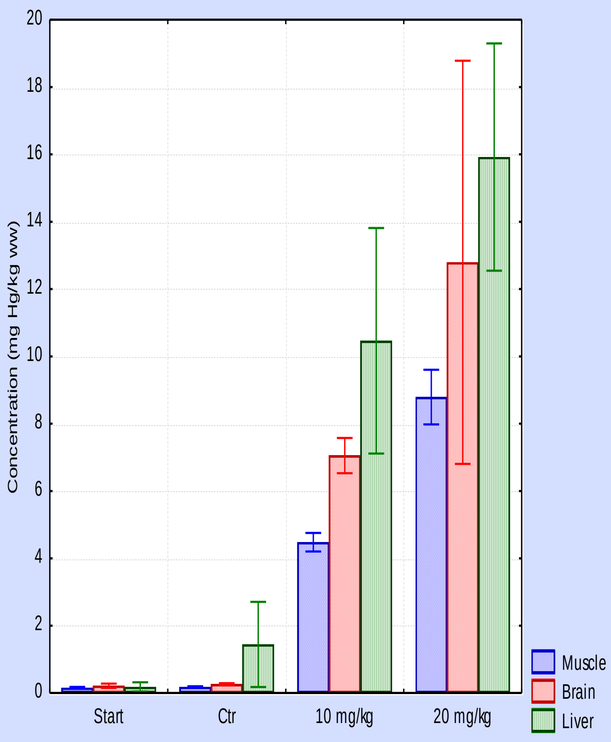
Introduction

Fish farming requires formulated feed which meets the nutritional requirements of the fish. However, feed ingredients may also contain environmental contaminants, such as mercury in fish meal. The present study used zebrafish (*Danio rerio*) as a model species to characterize assimilation and depuration of dietary methylmercury (MeHg) in adult fish, maternal transfer of MeHg to oocytes, and investigate the potential application of dietary selenium to reduce MeHg bioaccumulation.

Experimental outline

Quadruplicate groups per treatment (n = 25) of adult female zebrafish were exposed to dietary MeHg for six weeks followed by a four week depuration period. Methylmercury was added to a commercial zebrafish diet as methylmercury-cysteine at nominal concentrations of 0, 10 or 20 mg Hg/kg. After exposure and depuration, eight females from each tank were transferred to individual spawning tanks and paired with a male (non-exposed). After spawning, embryos were collected (sub-samples of 100 embryos) and pooled samples of brain, liver and muscle from three fish per tank were sampled for element analysis.

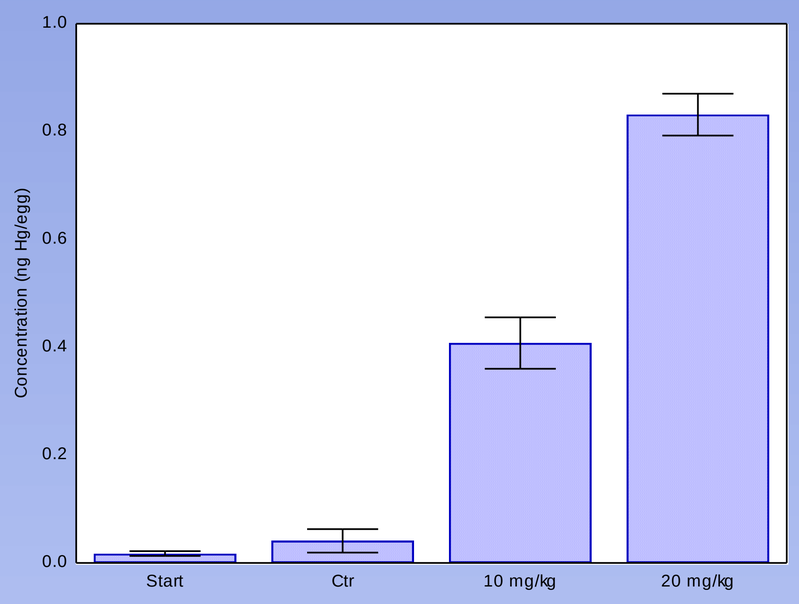
Accumulation of MeHg in liver, brain and muscle



Mean mercury levels (mg/kg ww) were determined in pooled samples of muscle, brain and liver (n = 4) of female zebrafish exposed to three levels (0, 10 and 20 mg Hg/kg) of dietary MeHg for six weeks. Samples were digested by microwave-assisted decomposition and the total mercury concentrations were determined by inductively coupled plasma mass spectrometry (ICPMS).

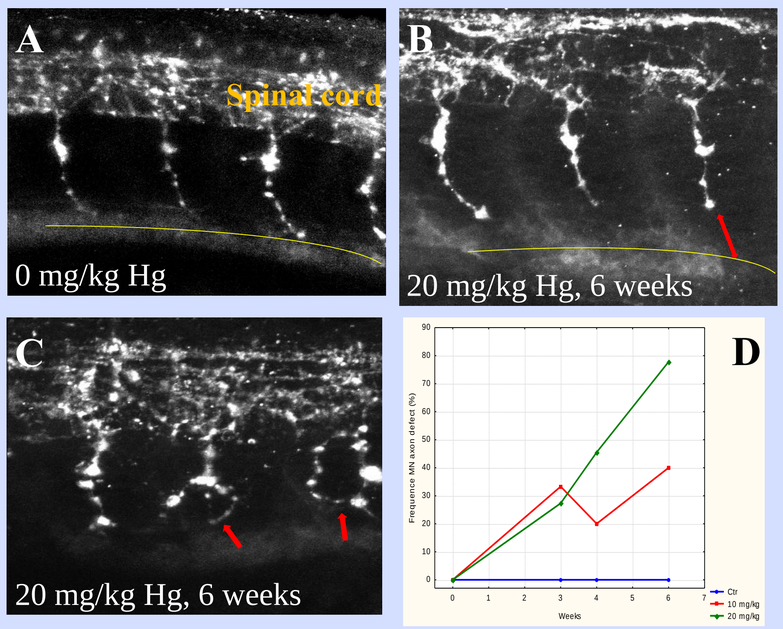
The accumulation of dietary MeHg was dose dependent in all investigated organs, with the highest tissue levels seen in zebrafish exposed to the highest dietary mercury level (20 mg Hg/kg). At both exposure levels the mercury concentration was higher in liver and brain than in muscle, indicative of the propensity of MeHg to cross the blood-brain barrier.

Maternal transfer of dietary MeHg in zebrafish embryo



Batches of 100 embryos from crosses of female fish fed 0, 10 and 20 mg Hg/kg for six weeks were analyzed for their mercury content using a direct mercury analyzer (DMA-80). Maternal transfer of MeHg was also dose dependent, with higher mercury levels found in embryos of females fed the 20 mg Hg/kg diet compared to embryos of females fed the 10 mg Hg/kg diet, with implications for embryonic neurodevelopment. Amounts are given as ng Hg/embryo.

Maternal transfer of MeHg leads to disturbance in spinal cord motorneuron axon growth in zebrafish embryos



Spinal cord primary motorneurons were visualized by immunohistochemistry (Znp-1 antibody) on batches of 10 embryos from each diet. We observed a significant disturbance in caudal primary CaP axon growth in embryos from females fed the 10 and 20 mg Hg/kg diets (D). Their axons projected to the branching point, but then growth ceased, leading to a shortened axon, or in other cases led to abnormal branching and misguided axon growth compared to unexposed control (red arrows, yellow line, A-C).

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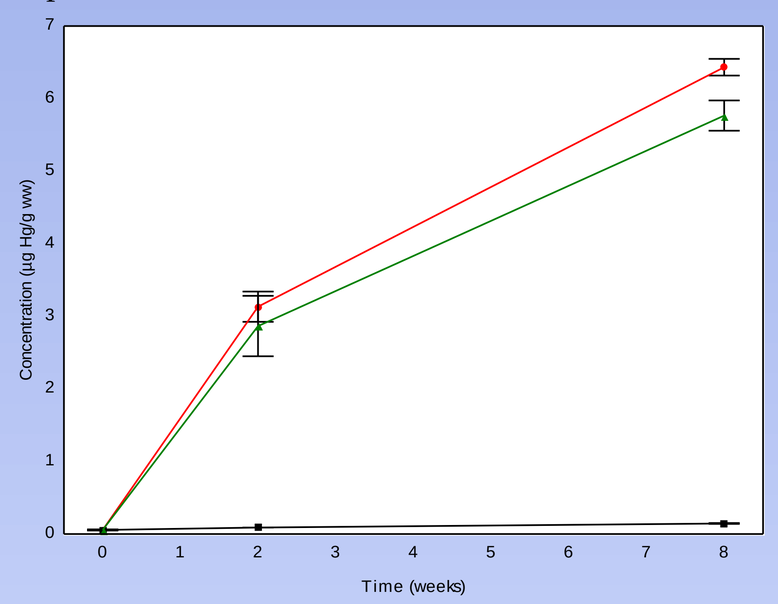
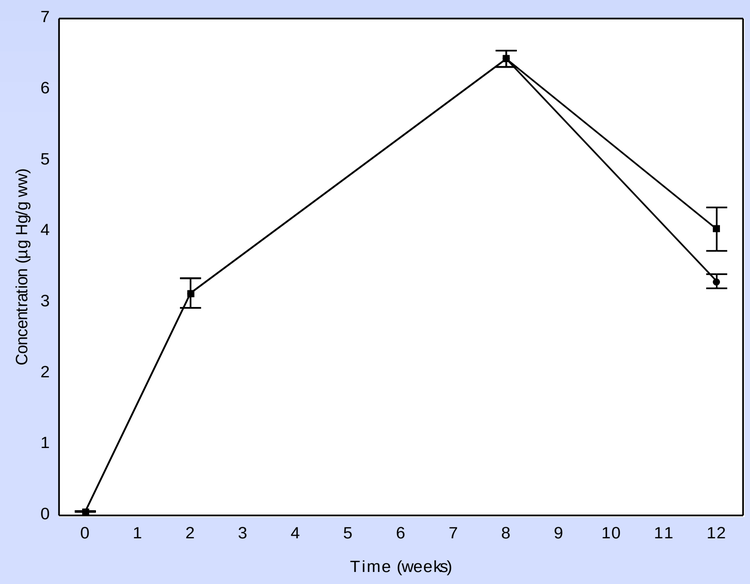
Dietary selenium influence the assimilation and elimination of dietary MeHg in muscle

In a second feeding trial. quadruplicate groups per treatment (n = 25) of adult female zebrafish were exposed to dietary MeHg with or without added selenium (as selenocysteine) for eight weeks followed by a four week depuration period. At weeks two, eight and twelve, three fish from each tank were sacrificed, and pooled samples of brain, liver and muscle were sampled.



Mercury levels (µg/g ww) in muscle of zebrafish (*Danio rerio*) exposed to 10 mg Hg/kg diet (red line), 5 mg Se/kg diet (black line) or 10 mg Hg/kg and 5 mg Se/kg diet (green line). The total mercury concentrations were determined ICPMS. The zebrafish fed MeHg and selenium accumulated less mercury than the fish fed only MeHg.



Mercury levels (µg/g ww) in muscle of zebrafish (*Danio rerio*) exposed to 10 mg Hg/kg diet for eight weeks. During a four week depuration period the fish were fed a diet containing 0 (■) or 5 mg Se/kg diet (♦). The total mercury concentrations ICPMS. The zebrafish fed selenium had lower levels of mercury in muscle at the end of the depuration period.

Conclusions

- The accumulation of dietary MeHg (as methylmercury-cysteine) in organs of adult female zebrafish is dose dependent, with higher levels found in liver and brain than in muscle.
- The maternal transfer of dietary MeHg is dose dependent.
- Maternal transfer of MeHg leads to disturbance in spinal cord motorneuron axon growth in zebrafish embryos
- Preliminary results indicate that dietary selenium (as selenocysteine) reduces accumulation of MeHg and enhances elimination from muscle